Protein family review

The ring-type polymerase sliding clamp family

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Summary

Ring-type polymerases consist of a DNA polymerase, a ring-shaped sliding clamp protein and a clamp-loading complex. Sliding clamp proteins are found in all organisms and are called proliferating cell nuclear antigen (PCNA) in eukaryotes and the β clamp in prokaryotes. Both PCNA and β form a ring around DNA, which is made up of two subunits of three domains each in β but three subunits of two domains each in PCNA. Despite this difference and a lack of detectable sequence homology, the structures of the two rings are very similar. The sliding clamp slides along DNA and tethers the polymerase to the DNA, enabling rapid and processive DNA replication.

Gene organization and evolutionary history

The ring-type polymerases (also called replicases) are found in all organisms and consist of three major components: the DNA polymerase, a protein ring or sliding clamp, and a clamp-loading complex. Their primary role is to replicate the genome. In this review, we focus on the sliding clamp proteins.

Classification

The prokaryotic sliding clamp is a protein referred to as β and the eukaryotic sliding clamp is called proliferating cell nuclear antigen (PCNA). The T4 bacteriophage also utilizes a ring-type polymerase; its sliding clamp, called gene protein 45, is a trimer similar to PCNA (see Figure 1), but lacks homology to either PCNA or β [1,2].

Gene organization

The location of the *dnaN* gene, which encodes the β sliding clamp protein, is conserved among prokaryotes. In both the Gram-negative and the Gram-positive genomes sequenced to date, the *dnaN* gene is embedded between the *dnaA* and the *recF* genes, within the replicative-origin region of the bacterial chromosome. Even though differences in the organization of bacterial origins have tentatively resulted in three classes of origins, the position of *dnaN* relative to *dnaA* and *recF* is conserved in all classes [3]. The promoter and the regulatory sequences of the *dnaN* gene operate from within the *dnaA* gene, but expression of the β subunit is independent of DnaA [4].

The final transcript of the human PCNA gene contains six exons. The PCNA gene has been mapped to chromosome 20, but two pseudogenes have been identified on chromosomes X and 6 [5].

Evolutionary history

The ring-type polymerases are found in all organisms, both prokaryote and eukaryote. The existing body of genome sequence information indicates that the β sliding clamp proteins are highly conserved in prokaryotes, and PCNA is highly conserved among eukaryotes. Interestingly, β and PCNA show no sequence homology, even though they have very similar three-dimensional structure [6]. Homologs to *Escherichia coli* β protein are readily identified in all the numerous prokaryotic genome sequences by simple BLAST searches. There is at least one example of an organism (*Sulfolobus solfataricus*) that encodes two β homologs, and others may yet appear. The PCNA sequence is fairly well conserved among eukaryotes. In general, there is only one gene encoding PCNA, but the organism *Daucus carota* (carrot) encodes two PCNA homologs, one of which is expressed only during embryogenesis.

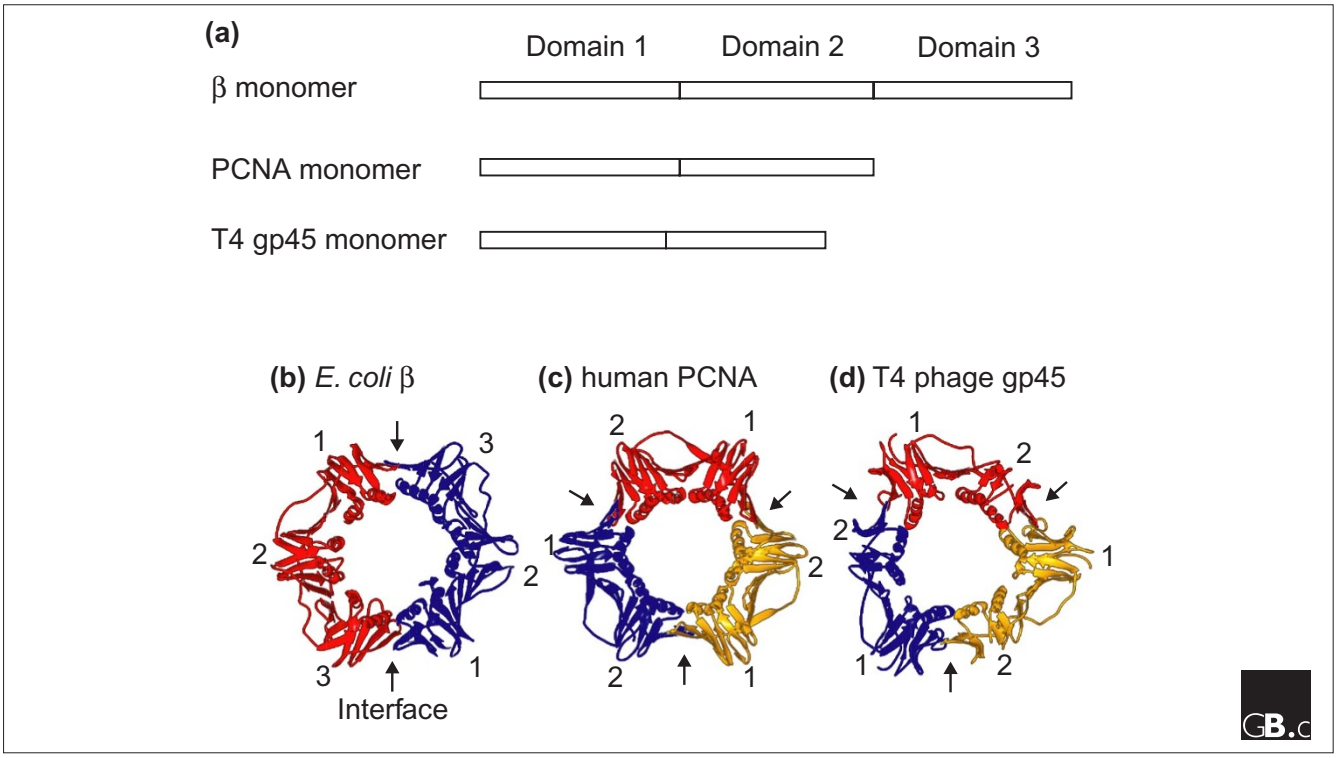

Figure 1
Sliding clamp rings of different organisms. Clamps are constructed from either two or three monomers to yield a ring composed of six domains. **(a)** The prokaryotic β subunit contains three domains, whereas PCNA and T4 gp45 are about two thirds the size of β and comprise only two domains each. The crystal structures of the oligomeric rings: **(b)** *E. coli* β; **(c)** human PCNA; **(d)** T4 phage gp45. In (b-d), the interfaces between protomers are indicated by the arrows, and the domains within each monomer unit are numbered (1-3 for β and 1,2 for PCNA and gp45).

Characteristic structural features

The sliding clamp is formed from identical protomers that oligomerize to form a ring that encircles DNA [7,8]. The ring does not self-assemble onto internally primed sites. Rather, it requires the clamp loader, which harnesses the energy of ATP hydrolysis to open the ring, to position DNA within it, and to close the clamp (Figure 2).

The β clamp of the *Escherichia coli* replicase is a homodimer of crescent-shaped 40 kDa subunits arranged head-to-tail to

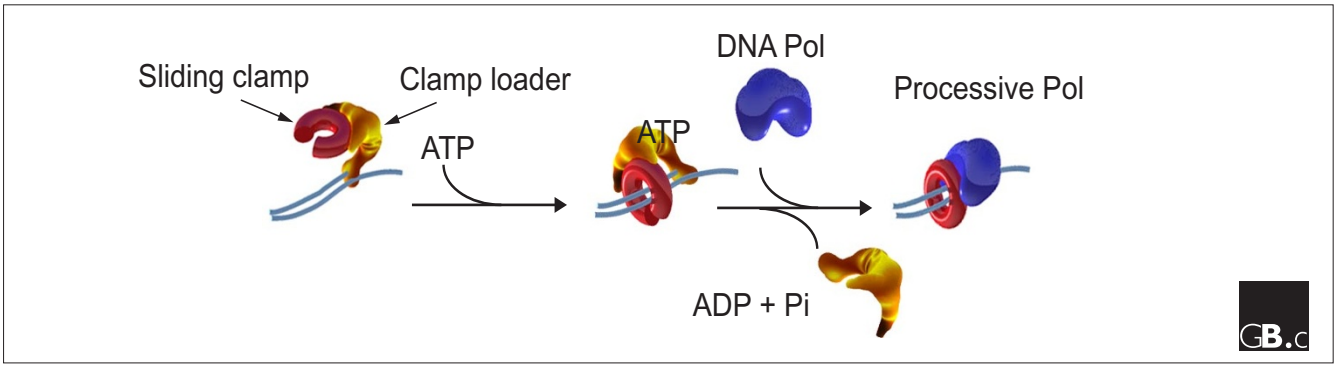

Figure 2
The action of the three components of the ring-type DNA polymerases. The protein ring (sliding clamp) is assembled onto a primed template junction by a clamp-loader complex in an ATP-driven reaction. The DNA polymerase (Pol) then assembles with the ring on DNA to form a highly processive polymerase, which pulls the ring along behind it during chain extension while remaining tethered to DNA by the ring.

form a ring (Figure 1). The crystal structure of β reveals that each monomer is constructed from three globular domains, each with the same chain fold [7]. The inside diameter of the ring of both β and PCNA is approximately 35 Å, allowing ample room to encircle the DNA duplex.

The structure of eukaryotic PCNA is practically superimposable on that of the β clamp [4,9]. The monomeric unit is only about two-thirds the size of β, however; it consists of two globular domains instead of three and trimerizes to form a six-domain ring the size of the β dimer (Figure 1). Although the PCNA domain structure is essentially the same as that of the domain structure in β, no sequence homology is detected between the two families. Perhaps the multidomain structure evolved from a common ancestral gene encoding one domain that later underwent duplications and fusion events to form the three-domain monomer.

Localization and function

The ring-type polymerases are ubiquitous in all cells. Their primary role is to replicate the genome [4,10,11]. They are highly processive enzymes and extend DNA at high speed. The DNA polymerase component is relatively poor in DNA synthesis because it dissociates from DNA after synthesis of only a few nucleotides (called 'distributive action'), and must rebind to DNA to continue synthesis. But when coupled with the sliding clamp and the clamp-loading complex, it becomes rapid and highly processive. The sliding clamp slides freely on duplex DNA [12] and binds directly to the DNA polymerase, thereby acting as a mobile tether to hold the polymerase to the DNA template during synthesis.

The ring-type polymerases are utilized for chromosome replication, but are also involved in other processes. For example, the *E. coli* DNA polymerase III holoenzyme is required in mismatch repair. In eukaryotes, PCNA is involved in both excision and mismatch repair.

Expression of the PCNA gene is associated with the proliferative state of the cell. The promoter sequence contains binding sites for several transcription factors. Transcription of PCNA is stimulated by a number of growth factors, so it is not surprising that the expression of PCNA is lowest in quiescent cells.

Frontiers

As the ring-type polymerases are involved in processes other than DNA replication, it seems likely that future studies will reveal how this three-component machinery interfaces with yet other proteins to perform its role, not only in replication, but in DNA repair and possibly recombination as well.

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